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THE SEROLOGY OF SYPHILIS WITH SPECIAL REFERENCE TO CHANGES IN THE TECHNIC OF THE KOLMER COMPLEMENT FIXATION TEST

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In the first place, permit me to express deep personal interest in the Texas State Institute for Laboratory Technicians, as I believe this is a sound and highly desirable method of post-graduate instruction for the purpose of enabling and encouraging technicians to keep informed of developments in laboratory methods and diagnosis. Throughout the United States physicians have found graduate assemblies or institutes of great value in keeping abreast of advancements in medicine and surgery and I hope that the American Society of Clinical Pathologists will foster and encourage this kind of thing in relation to laboratory technicians, which has been inaugurated with so much success by your Institute under the aegis of the University of Texas.

Under the circumstances I particularly appreciate this opportunity for directing attention to a few changes in the technic of my complement fixation test for syphilis which I believe have resulted in increasing its sensitiveness while maintaining a high degree of specificity and particularly since it is generally admitted that laboratory examinations continue to be the chief means of diagnosis of

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this disease at the command of the large majority of practitioners of medicine. For the relatively few expert syphilologists, clinical training and experience in diagnosis and treatment reduce the demand and necessity for laboratory aids, but even under these circumstances everyone skilled in syphilology must admit that in the diagnosis of concealed or so-called latent syphilis and as a guide in treatment, the serological tests are indispensable. Certainly the history alone of not a few victims of the disease may be notoriously misleading and in the latent or concealed stages coincident with good general health its detection by clinical methods alone may readily escape the most expert. No one can even calculate with reasonable accuracy the incidence of unsuspected syphilis, and for the detection of these, the various serological tests have played and must continue to play a very important role.

It is sometimes stated that the diagnosis of syphilis should never be made in the laboratory; on the contrary, it is easily proved that in not a few instances the diagnosis of primary or acute and chronic syphilis can be made only in the laboratory. I may state therefore on the basis of both clinical and serological experience, that the laboratory tests for syphilis must continue to play a very important part in both diagnosis and treatment with the great majority of both general practitioners and specialists in medicine and surgery.

Unfortunately, many laboratories and technicians do not enjoy the privilege of close contact with experts or clinics devoted to syphilology for checking the results of their serological work. This places upon them an added responsibility and necessity for the most accurate work with special reference to a choice of method or methods to be employed. Furthermore, the great majority of reports are sent to general practitioners who, under the circumstances, must accept the results and give them great weight in arriving at diagnosis and arranging therapeutic programs. A mistake in serology in either a positive or negative way may be of no great consequence to the expert syphilologist but, to the less experienced physician, it may be and usually is a serious matter sometimes associated with tragic consequences. I repeat, therefore, that all serologists and laboratories engaged in the serum diagnosis of syphilis assume a great responsibility and especially as it represents the hope and only means at the command of a large number of physicians for the aid so urgently required in the diagnosis of syphilis among indigent individuals and those who cannot afford the services of the specialist.

Of importance to all laboratories, but more especially in the case of those without any detailed information concerning the

clinical status of patients, is the question of the specificity of the serum reactions for syphilis with particular reference to weakly positive and unexpectedly positive reactions. During the active stages of syphilis with large amounts of antibody in the blood, most any of the numerous serological tests are apt to yield positive reactions and present no difficulties in diagnosis; but in concealed and unsuspected or doubtful cases the serum tests should possess above everything else a reliable practical specificity for syphilis so that proper reliance may be placed upon all positive reactions including those that are but weakly and unexpectedly positive. Under these circumstances it is proper to place the burden of proof on the clinician rather than on the serologist when the question of diagnosis is raised or disputed, and require the former to prove that syphilis is absent rather than to require the latter to prove that it is present or to seek various technical reasons and excuses for the positive reports.

Every experienced serologist knows that both complement-fixation and precipitation procedures may be made too sensitive with the risk of securing non-specific or falsely positive reactions with the sera of non-syphilitic individuals. Personally, I would much rather run the risk of securing an occasional falsely negative reaction than a single falsely positive one, and I think this attitude and practice is especially befitting those laboratories without the benefit of close contact with the expert syphilologist for the final evaluation of disputed results. What is particularly needed by all is a test or tests possessing the maximum of specificity consistent with sensitiveness in order to earn the confidence of the medical profession in the significance of positive reactions and to teach the great risk of error and responsibility involved in ignoring weakly positive reactions if they happen to be unexpected or out of harmony with preconceived clinical diagnosis. Therefore, the test or tests employed for the serum diagnosis of syphilis should possess the maximum of sensitiveness consistent with specificity because in the majority of instances it is in the concealed or doubtful case or treated case of syphilis with but small amounts of antibody in the blood that the aid of serum diagnosis is most urgently required.

In other words, while complement-fixation and precipitation tests serve a useful purpose in confirming the clinical diagnosis of syphilis, yet there can be no denial of the fact that they should be made especially valuable and available for the diagnosis of unsuspected or concealed cases when diagnosis is difficult or impossible by other means.

Furthermore, the maximum of sensitiveness consistent with specificity is required when serum tests are employed as guides in

the amount and kind of treatment to employ. To the best of my knowledge syphilitic infection is to be regarded as present as long as reliable tests yield truly positive reactions. In some cases it may not be possible or even desirable to administer sufficient treatment to secure permanent negativity on the part of serum reactions, but yet it is to be realized under these conditions that infection persists and to take the necessary therapeutic measures and precautions which are beyond the scope of the present discussion. Indeed it would appear that syphilis is not being over-treated; rather it is to be feared that in many cases it is being under-treated and the laboratory can do a great deal in the prevention of this very regrettable error by employing a test or tests possessing the maximum of sensitiveness consistent with specifically and encouraging their use as serological guides in treatment.

With the large number of modifications of the Wassermann and the various precipitation tests now being advocated, I can well understand the perplexity experienced by technicians in the choice of method or methods to be employed.

In some instances and especially during the present difficult financial conditions, a decision may be based more upon the necessity of exercising the strictest economy than upon scientific truth or the best service. This is right and proper if not pushed to the extent of seriously involving the questions of specificity and sensitiveness. Personally I believe that it is better not to employ the serum tests at all, but to rely upon clinical judgment alone in the diagnosis and treatment of syphilis, if the serum tests are lacking in a reasonable degree of accuracy and sensitiveness.

Without doubt the precipitation tests are more economical in materials since a hemolytic system is not required, but they are hardly more economical in time as compared with complement fixation tests and certainly require an equal degree of skill and experience for their conduct and especially when it involves the matter of reading doubtful or weakly positive reactions. There can be no doubt about the importance of the latter; that is to say, a serum containing large amounts of antibody and yielding strongly positive reactions and one containing no antibody at all and yielding completely negative reactions offer no difficulties in reading and interpretation, but those containing small amounts of antibody and yielding weakly positive or doubtful reactions are the ones presenting most difficulty and are most subject to error and at the same time likely to be of most clinical importance and most urgently requiring the diagnostic aid of serum tests.

It may be that circumstances compel the adoption of a precipitation test alone for the serum diagnosis of syphilis, but I be-

lieve that a careful and impartial review of the situation amply confirms the prevalent opinion that the serum diagnosis of syphilis is still best served by employing both a complement-fixation and a precipitation method possessing the maximum of sensitiveness consistent with specificity as neither alone will detect as many cases of syphilis as the two together applied to each serum and that neither alone will exclude syphilis with as much accuracy as when both yield negative reactions.

In this connection I may state, however, that differences in the degree of positiveness should not be allowed to be a disturbing factor. A strongly positive Wassermann reaction may occur with a weakly positive precipitation reaction or the reverse. The only thing that really counts is specificity or the reliability or the positive reactions without regard to the degree which is so largely at the mercy of technical factors. Furthermore, there can never be a strict correlation between the degree of positiveness of either complement-fixation and precipitation tests and the clinical status of the individual patient since symptoms largely depend upon the physiological importance of the tissues infected while the degree of positiveness largely depends upon the degree of spirochetic activity. For example, a case of locomotor ataxia may present pronounced signs and symptoms with but weakly positive reactions, whereas a case of concealed syphilis with a heavy infection involving an organ or tissue of lesser importance may be in excellent general health with strongly positive reactions.

In the case of those laboratories required to conduct a large number of tests, I believe that it is a good routine practice to first conduct the Kline exclusion test on all sera since those giving negative reactions may be safely reported upon without the need of additional tests and thereby saving both time and expense. When properly conducted, it is very sensitive although it does not always detect cases of syphilis as occasionally a serum giving a negative Kline reaction will give a positive Wassermann reaction. But positive Kline reactions and especially those that are weakly positive, should be checked by a reliable complement fixation or some other precipitation test to guard against the regrettable error of falsely positive reports as, in my experience at least, the Kline exclusion test is so sensitive that it should not be relied upon alone for the serum diagnosis of syphilis.

Since first described (1) in 1922 my complement-fixation test has undergone a few changes which I believe have resulted in some improvement in sensitiveness while maintaining the high degree of specificity which has ever been my endeavor. These are fully de-

scribed in the new edition of "Approved Laboratory Technic," by Boerner and myself, to appear in early autumn, but which have been described elsewhere and briefly referred to herewith.

1. A change in the amounts of serum employed (2) which are now 0.2, 0.1, 0.05, 0.025 and 0.005 c.c. with 0.2 c.c. in the serum control in the qualitative test or 0.2 and 0.1 c.c. with 0.2 c.c. in the serum control in the qualitative test. These larger amounts of serum as suggested by Boerner and Lukens have definitely increased the sensitiveness of the test with no appreciable increase of anticomplimentary or non-specific reactions. Cerebrospinal fluid is employed in the same amounts as originally advised.

2. The use of a new C. L. antigen (3) re-enforced with acetone insoluble lipoids in dose of 20 instead of 10 antigenic units. This antigen in its larger dose has proven quite satisfactory in Philadelphia but a few reports from different parts of the country have indicated that it may be too sensitive and for this reason the original C. L. antigen is still regarded as quite satisfactory.

3. A new method for determining the optimum dose of antigen to employ, as described by Boerner and Lukens, (4) which is probably better than that originally described. I am now convinced that it is unnecessary to determine the hemolytic and anticomplementary units of the original or new C. L. antigens and the method proposed by Boerner and Lukens for determining the antigenic or optimum amount to employ in the conduct of tests by my method is based upon sound principles and to be recommended. It consists of using different amounts of antigen with varying amounts of any strongly positive serum, the technic being described elsewhere (5).

Otherwise, the technic of my test may be exactly as previously described (6) and I can only hope that technicians will adhere strictly to every detail. Unfortunately, there is always the temptation to change technic here and there with the thought that it makes little or no difference in the results, but this is always apt to be unfair to any method. Indeed, instances of such changes have been brought to my attention where I could scarcely recognize the method as my own at all and it is well and wise for technicians to always faithfully follow any method exactly as described until changes are adopted or approved by its author.

In conclusion, I highly recommend lyophilized complement which is a method of dehydrating guinea pig serum at a very low temperature according to the method of Flosdorf and Mudd (7). By this process it is possible to pool the sera of a large number of pigs and preserve them very satisfactorily in a refrigerator for periods up to a year without loss of hemolytic activity or fixability

by antigen and antibody. This enables one to prepare all the complement required for a year, at a time when guinea pigs are cheapest and during the cooler months, which not only increases the sensitiveness and accuracy of complement-fixation tests, but also is economical in time and expense as well as reducing to a minimum unsatisfactory reactions due to defective complement, which are particularly apt to occur during the hot summer months.

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WHAT THE REGISTRY HAS DONE FOR THE MEDICAL TECHNOLOGIST

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The eminent historian, Van Loon, has said, "We live under the shadow of a gigantic question mark." Thus, it is with the Registry. What is the Registry? What is its aim and purpose? Its scope and requirements? These and many other questions are answered in the Registry literature. In this paper we shall consider—What has the Registry done for you, the Medical Technologist?

As you all know, the Board of Registry was established in 1928 by the American Society of Clinical Pathologists. Since those early days, the Registry has gradually but in less than a decade established order and a program for the future. When the work of establishing and maintaining standards of educational and technical qualifications was inaugurated, it was found that many of the individuals engaged in laboratory work were inadequately trained with resultant injury to patients and discredit to competent workers. No idea whatsoever was prevalent as to what education and instruction a laboratory technician should have. Besides the irregular methods of training that did provide able technicians, there were many short courses, usually under questionable direction, being offered students irrespective of their academic background. No attention was given to those personal and psychological attributes now recognized as not only desirable but necessary. While true that some individuals were accorded the respect of other workers in the medical world, the majority found their status most uncertain. Whether the technician had a highly specialized technical training or only a smattering of technic in the more obvious laboratory procedures, he or she claimed the title of laboratory technician. Many of the latter little realized their own shortcomings, while the competent person resented inclusion in the same category.

Since its inception the Registry has invested this useful calling with the dignity it deserves and created a proper spirit of appreciation for the members whose qualifications are vouched for by the Registry certificate, and whose work is primarily dedicated to the

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aid of human sufferers. In short, through this certificate of competence, the Registry has invested you with a profession. It has improved your scientific, economic, and social status. You pioneers will recall the lifted eyebrows, the blank expression when you said you were a laboratory technician. No longer do you hear the "What is that?" query. Indeed, so far has the Registry spread the gospel that today even the high school student is well aware of the purpose of the Registry. Some months, the Registry receives hundreds of inquiries from such students asking for the best advice in preparing for the vocation of a Medical Technologist. No longer a vague something, today students know there is a standardized course of training and that there is no short or easy road to knowledge and no open sesame to clinical, hospital, research or public health laboratories other than that established by the Board of Registry.

We repeat that the Registry has given you, instead of an itinerant job, a profession with a background of which you can be justly proud. Not only the clinical pathologist, but the hospital superintendent, staff doctor, and all others connected with the health program, even the laity, know that your role as the indispensable assistant of the clinical pathologist is equally important with that of the nurse who serves the patient under the attending physician. Your scientific status is attested by the educational, scientific, and technical knowledge required for all those certified by the Registry as well as by your connection with the scientists of medicine, the clinical pathologists.

There is a significant advancement in the economic and social status of the registered Medical Technologist. While present general economic conditions are to some extent responsible, the activities of the Registry have also brought about an improvement, not alone by controlling the number of workers in the field, but through educating the profession to the need for competent technologists. We shall avoid statistical data in this paper but might mention a recent cursory compilation of figures on salaries which pointed to an advance in the average monthly compensation of our registrants of about 20%. Daily correspondence evidences a constantly increasing demand for registered Medical Technologists and a wider recognition of their rightful role in medical institutions. Probably you can remember when the hierarchy of the hospital family excluded the "lab. girl" from the nurses' table in the dining room. How many sad stories we heard five or six years ago. Today to a great extent due to the good influence of the Registry, the Medical Technologists can claim their rightful place. To reminisce further, just a half dozen years ago an M. T. was unknown. Today due to the Registry, you have a specific title denoting an honorable and re-

spected calling in the medical field, accepted by the large medical bodies of the country, the medical profession in general, and even the general public. Like the M. D. or the R. N., the M. T. is now a recognized entity.

While it is not the intention to claim undue credit for what has been accomplished, I am confident you will agree that no such transition could have been made from an unrecognized status to that of a full fledged M. T. in so short a time and so well under other auspices. The members of the Board of Registry come from various sections of the country. They are familiar with local as well as national conditions. They, also, have the technologist's best interests at heart. They have founded and developed the Registry step by step with careful forethought and planning. Changes have been made from year to year but always toward the goal of higher standards. An attitude of impartial and thorough consideration of all the problems that affect the Medical Technologist has prevailed without prejudice as to race, creed, nationality, or regional conditions. The Medical Technologists themselves could not have laid down or executed such a program. In spite of all endeavors to free an organization of workers from personal prejudice and ulterior motives, we human beings are so constituted that there always arise contentions and petty differences, blocs form and one faction or another defeats or delays the best laid plans which are intended for the benefit of all.

As the Registrar, I hope it will not be amiss to mention the fact that a kindly Providence has decreed that the clinical pathologists who have and are serving on your Board of Registry and who are responsible for the establishment of its principles, are men of wide interests and broad experience in the field of medicine and its allied problems; and are even more interested in you and your problems than most of their colleagues. The Chairman and Secretary of the Board since its inception have always been keenly concerned with your careers individually and collectively. They have given unstintingly of their time and help, often at a personal sacrifice, without any reward other than the satisfaction of accomplishment. The same applies to the other members of the Board, each of whom, unlike most committee members, carries out a special assignment for the Registry each year. These doctors are responsible for the present permanent organization which can in no way be construed as a temporary unstabilized certifying body. They have builded for the future security of the Registry and its precepts. Not only is permanence assured from a scientific standpoint but from a sound financial basis as well. The day is approaching when new applicants will be limited and the registration fees which now

provide the main funds for conducting the Registry will be comparatively small in amount. Provision, however, has been made for the rainy day in the form of a trust fund assembled from the annual residue of moneys from past years when the expenses were at a minimum.

With the increased work, the need for more help in the office, more equipment, the introduction of examinations, etc., the cost of conducting the Registry now just about parallels the income. To do this requires careful checking of all expenses.

For instance, the annual renewal dollar is utilized as follows:

January 1st letter, statement, postage, etc.....	.07
Certificate, registration card, and incidental expenses34
Roster and incidental expense.....	.24
New edition of Registry booklet of information, mailing, etc.07
Direct clerical service per registrant.....	.26
Total.....	.98

Some other interesting items that indicate the scope and size of the Registry's activities during 1936-37 were:

Advertising	\$ 318.44
Convention and Exhibit Expenses.....	999.58
Postage	1,623.58
Marking Examination Papers.....	371.50
Registration fees refunded.....	677.00
Rent	495.30
Salaries	4,265.00
Stationery, Postage, and Office Supplies.....	3,190.00
Telephone and Telegraph.....	102.67
Other expenses	1,093.90
Total.....	\$13,136.97

The persistent and concerted action of the Board has also been responsible for the recognition of the registered Medical Technologist on the part of the American Medical Association, the American College of Surgeons, the American Hospital Association, and other medical bodies. The October 1936 issue of the Bulletin of the

American College of Surgeons states: "It has been gratifying to note continued improvement of the technical service in the laboratories in approved hospitals, achieved mainly through the registration of laboratory technicians by the Registry of Technicians of the American Society of Clinical Pathologists. This organization has standardized the training of laboratory technicians and today every approved hospital should have only those who are registered."

Not only the staff inspectors of the College of Surgeons but those of the American Medical Association recommend the employment of technologists certified by the Registry, and in their investigations require the Medical Technologist to exhibit his or her Registry certificate.

Through the combined and unceasing vigilance of the Registry and the Council on Medical Education and Hospitals of the A.M.A. the campaign to eliminate the so-called commercial school is bringing results. No longer are hordes of such graduates turned loose every six months. The Registry and the Council are likewise continuing their work of passing on the competence of training schools, thus insuring the registered M. T. protection against competition from poorly trained and "cheap" laboratory help. Most prospective students now seeking advice through the Registry are turned away from these schools. Those without the proper educational prerequisites are discouraged from entering the field. 6,000 hospitals were circularized twice during the past year by the Registry calling attention to the evils that accompany the interne or volunteer technician practice. This tends to replace paid technologists with incompetent workers, who are without a doubt a potential source of danger to the patient. Clinical pathologists, hospital administrators, and the medical profession in general have been solicited with respect to the employment of qualified registered M. T.'s for the good, not only of the doctor and the patient, but in justice to the well trained technologist who merits consideration on his or her own qualifications.

The Registry has cultivated a high ethical standard among Medical Technologists in accordance with its Code of Ethics. Through the genuine and moral support of our registrants many untoward practices and conditions have been eliminated under this Code.

High school counsellors, college vocational directors, social service agencies are all looking to the Registry as the authentic source of information in advising their students, so that even the potential technologist of future generations is already enjoying the benefits of the Registry.

Of late, zealous Medical Technologists have been misled by the plan of state licensure which seems to have arisen in some states. This policy has already been discussed at length. A copy of the article on the "advantage of the voluntary system over the legislative measures" has been sent to every registrant. State licensing for M. T.'s has been considered by the A. M. A., and the Council has made a definite pronouncement that such a step would not "represent any advance over present methods of control in this field." To adopt such a system would most certainly increase the cost of registration to the M. T. and would probably avail little or nothing in view of the fact that the hospital standardization program would not recognize the lowered standards of state licensure.

The Registry has endorsed and fostered all attempts on the part of local, state, and national groups of Medical Technologists to organize for the purpose of furthering their own best interests. In fact these societies are the direct outgrowth of the registration of laboratory workers.

The Registry has assisted the individual Medical Technologist in his or her problems such as supporting them in their refusal to teach technical procedures to proteges of staff doctors, hospital administrators, etc., in aiding them to secure new positions, in disseminating information on technical questions and in a variety of other ways. It has been instrumental in having the larger professional employment agencies give preference in their placements to registered M. T.'s. In fact their application blanks carry the question: "Are you registered by the Registry of Medical Technologists of the A. S. C. P.?"

After many delays the Registry has at last been notified that the seal and emblem of the Registry will shortly be reserved by trademark under the United States Patent Office for the exclusive use of the Registry and registered Medical Technologists.

The future plans of the Board include even greater advancements, such as enlisting the cooperation of universities and colleges for training M. T.'s under their auspices, also providing uniform pre-training college courses, post-graduate work, establishing seminars in various sections of the country, also underwriting a supplement in the "American Journal of Clinical Pathology" for Medical Technologists who desire to keep up with the newer methods and to review recent developments in technical procedures.

To summarize briefly what the Registry has done for the Medical Technologist:

- (1) It has established and is maintaining standards for those engaged in this field of scientific activity, and is certifying those qualified under these standards.
- (2) It has established adequate training in medical technology in recognized and reputable institutions under proper auspices.
- (3) It has given the M. T. a professional standing and improved his or her scientific, economic, and social status.
- (4) It has safeguarded their interests and promoted their welfare by eliminating the possibility of factions both within and without their own ranks.
- (5) It has provided an impartial competent organization for promoting this program.
- (6) It has enlisted the support of the influential medical bodies in furthering these principles.
- (7) It has provided an adequate Code of Ethics.
- (8) It is endeavoring to eliminate the control of registration of M. T.'s by sectional supervision of varying standards.
- (9) It has fostered local and national societies of M. T.'s for their own advancement.
- (10) It has established an official title and emblem identifying the M. T.
- (11) It has formulated a definite program for the future.

The Board of Registry is fully cognizant of the important part played by the registered Medical Technologist in establishing and furthering the program of the Registry, and desires to express their appreciation for the loyal support and enthusiastic cooperation of each and every M. T. who carries the Registry certificate. Your continued allegiance is sought to promote and further advance this worthy cause.

In closing, it might be of interest to the Medical Technologists present to have a glimpse of the office of the Registry, and we have therefore brought along a couple of lantern slides.

We extend a cordial invitation to each and all of you to visit our headquarters in Denver. Should your travels take you through the land of the Rockies you can count on a hearty welcome.

AN EXCELLENT MEDIUM FOR SLIDE CULTURES OF PATHOGENIC FUNGI

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The medium to be described is considered an excellent medium for cultivation and study of pathogenic fungi. It gives good growth, demonstrates characteristics typical of the organism and brings out these characteristics so that they may be easily seen and followed. It allows growth in a comparatively short time and inhibits growth of certain other organisms.

The medium consists of $\frac{1}{4}\%$ d-glutamic acid, 4% dextrose, $1\frac{1}{2}\%$ agar, $\frac{1}{8}$ to $\frac{1}{4}\%$ eosin Y and is adjusted to pH 5.6. Other amino acids may be substituted for d-glutamic acid as may other acid dyes in appropriate concentration for eosin Y. Any slide culture method may be used; the hanging-drop method seems preferable since moisture in the medium can be better maintained and structures of differential importance seem to develop somewhat better.

Dyes in order to be valuable must be neither too concentrated or too dilute. Acid dyes are preferable because of lack of toxicity. Basic dyes may be used if in sufficiently dilute solution. For example 0.001% methylene blue shows dye concentration at and in certain growths, color change and evidence of reduction (1).

As early as 1925 eosin (2) in Sabouraud's medium was reported as an innocuous vital dye for some five pathogenic fungi. Methylene blue was considered too toxic. This is not so if methylene blue is sufficiently dilute.

We (3) have emphasized the importance of dyes in mediums in contrasting and bringing into relief important colony characteristics. Reduction of dyes by pathogenic fungi (1) and selectivity of the various parts of the organism for the dye have also been studied. Subsurface growth and the localization of the dye in such growth (4) is furnishing an invaluable tool for study in physiology and diagnostic features of fungi imperfecti and may aid in determining whether they possess a sexual life.

Observations of representative Achorion, Epidermophyton, Microsporons and Trichophyton suggest many possibilities. For example, spore capsules seem to vanish leaving an amorphous material which may be of sexual nature. This material seems to

collect about or is exuded from certain spores. The dye emphasizes such features as sprouting of mycelia from spores and conjugation of the various elements of the growth.

There is a suggestion that the lower percentage of eosin yields larger and more of the sac like spores. The consistency of this feature has not been determined. Frequent studies of the slide cultures should be made since in some organisms there is sufficient concentration of eosin with age to shadow some of the differential details. This concentration may be due to alkalinity developed in the growth. Since eosin is an acid dye it would be attracted to the more alkaline area.

In conclusion, a new medium for slide culture of pathogenic fungi is described and its possibilities discussed. It is synthetic and a number of comparatively cheap amino acids on the market make it practical. Eosin for bringing out differential features is discussed but other dyes may be used. At present, study is in progress in which methyl blue and eosin are being incorporated in the same medium.

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NOTE—Peptone, dextrose and agar from Difco Laboratories, Inc.
d-glutamic acid from Hoffman-La Roche & Company.
Eosin Y (NE 11) from National Aniline and Chemical Co., Inc.

DISCUSSION OF ALLERGY TECHNIQUE

By DOROTHY POLLARD MORRIS, M.T.

Newport News, Virginia

In 1856 Charles Harrison Blackley of Manchester, England, started some experiments, chiefly upon himself, that in later years came to be known as allergic studies. He was a sufferer from hay fever and was seeking methods of relieving himself, realizing the fact that there must be some external substance causing his symptoms. He made the first skin test by applying some dry pollen in an abrasion on his arm which caused a rather violent reaction. He did the first intranasal test by applying some dry pollen to his nose and reproducing his symptoms. He made the first pollen count by exposing slides coated with glycerine to the outside air and then demonstrating them under the microscope. He showed further that the severity of symptoms usually were dependent upon the amount of pollen in the air and that this pollen was dependent upon weather conditions.

In 1906 the term "allergy," which briefly means an altered state of activity on the part of an individual, was coined by von Pirquet and his associates. An individual who is said to be allergic is sensitive to something or reacts to some substance which the so-called normal individual does not react to and therefore is said to be in a state of altered activity or allergic.

In 1912 Schloss reported a child sensitive to buckwheat and demonstrated it by a skin test. From this time on the use of the skin test has taken an important place in the diagnosis of sensitivity to foods, pollens and other substances giving rise to allergic disease.

The purpose of the skin test is to find out what substances are causing patients to have allergic disease. The most important allergic diseases are asthma, hay fever, urticaria, eczema and migraine. There are however many other conditions in the body which may be caused by sensitivity to foreign protein substances. While the skin test has become the most accepted mode for finding out which substances are causing trouble, there are many other methods of approach to establish these causes which do not come within the scope of this paper to discuss. I shall confine my remarks to a discussion of the methods concerning the technologist.

Read before the American Society of Medical Technologists at Atlantic City, N. J., June 9, 1937.

1. Scratch or cutaneous test.
2. Intradermal or intracutaneous test.
3. Ophthalmic test.
4. Patch test.
5. The passive transfer test for Prausnitz-Kustner.
6. Leucopenic index.

The cutaneous or scratch test is used for testing with foods, epidermals, bacteria and pollens. The powdered extracts are usually obtained from a commercial house. They may be applied as such, by adding a little N/10 sodium hydroxide to the area, or the solution made from them may be applied. This solution which is made by adding 50 milligrams of powdered extract to 2 c.c. of Stier-Hollister solution simplifies the test considerably, because it is much easier to handle than when using the powdered allergen with sodium hydroxide and there is less danger of getting them mixed. Also, the solution does not dry out before the skin has had time to absorb enough of it to produce a reaction. The solutions are kept in 2 c.c. bottles with rubber caps and are arranged on a board in rows of 10 to 12. The solutions will keep indefinitely by constant refrigeration at 45 degrees F.

The foods, epidermals, and molds for the scratch test are put in rows on the patient's back. The number of scratches in a row corresponding to the number on the board. The scratches are made with a dull knife, preferably a chalazion knife, which is just sharp enough to break the skin and not sharp enough to cause the wound to bleed. Drawing blood makes it uncomfortable for the patient and tends to wash the extract away. The scratches are made at least 1 inch apart and $\frac{1}{2}$ inch in length. The size of the scratches should be uniform as the dimensions of the incision are factors in the reaction. Approximately 12 rows can be put on the patient's back. To each scratch apply a small amount of the allergen with a tooth pick and gently rub in. Do not make scratches where there is already an allergic reaction such as hives or eczema. Do not make tests if patient has had epinephrin or ephedrine for the relief of his symptoms within a period of 12 hours prior to test. Always select normal areas of skin for performing skin studies.

Without any exception pollens are always put on the arm over the flexor surfaces of the arm and forearm. These scratches should be made 2 inches apart and sufficiently below the armpit to permit the application of a tourniquet if necessary.

All skin tests are read in $\frac{1}{2}$ hour, 6 hours, and 24 hours routinely. Sometimes a reaction will appear in 10 minutes and

fade out in $\frac{1}{2}$ hour. This should be noted at the time it appears and a record made thereof. Reactions are read by the clinician and recorded by the technologist.

The intradermal or intracutaneous method of skin testing is a much more sensitive test than the scratch method and is made as a follow up of the scratch test. The extracts may be made in the laboratory as a great many allergists are doing or they may be bought commercially. The most convenient syringes are the blue-barreled tuberculin type of one cubic centimeter capacity and graduated in hundredths of this amount. The needles used are 26 gauge, stainless steel with a short bevel. The skin is sterilized with alcohol and exactly two one-hundredths of a cubic centimeter of the allergen is injected intradermally. If the allergen has been injected correctly a raised wheal will appear about 4 mm in diameter. Space the injections at least three inches apart so that in the event of a reaction the two areas will not coalesce. Positive reactions usually appear in 15 to 30 minutes. They appear as raised wheals much larger than the non-reacting wheals and often have irregular outlines and long pseudopods. The tests are read in the same time intervals as the scratch test.

It is very important for the technologist to be aware of the possibility of constitutional reactions in the patient while skin tests are being done and to take such necessary immediate precautions to facilitate the treatment thereof. On the appearance of any unusual symptoms such as sneezing, coughing, itching of the palms, appearance of urticaria or hives and so forth the report should be made immediately to the clinician. In doing intradermal tests, one should always have at hand a bottle or an ampoule of fresh 1/1000 adrenalin and sterile syringe available for the clinician. Patients should remain in the office for at least one hour after the tests have been completed.

The ophthalmic reaction is often useful in allergy when skin tests to pollens are negative. To make this test place a minute amount of the dry pollen by direct application to the conjunctiva. This test however has its limitations as to number of allergens and the temporary disfigurement of the patient in the event of a positive reaction.

The patch test is employed when it becomes necessary to test with specific face powders, soaps, fur from a coat or collar, felt from a hat and any substance that may be the cause of a contact allergy. Place a small amount of the substance to be tested on the back if possible, diluting it with sodium hydroxide, plaster down with adhesive plaster and leave for 24 to 48 hours. We have

found that simply moistening the substance with distilled water is sufficient.

Sometimes it becomes expedient to do a passive transfer study which is accomplished very easily. A small amount of the serum from the affected individual or patient is injected intradermally into a non-allergic individual and some of the substance which has been suspected is applied by either the scratch or intradermal test. If the patient is sensitive a positive reaction will develop. The test however is rather impractical and in our work has just about been abandoned.

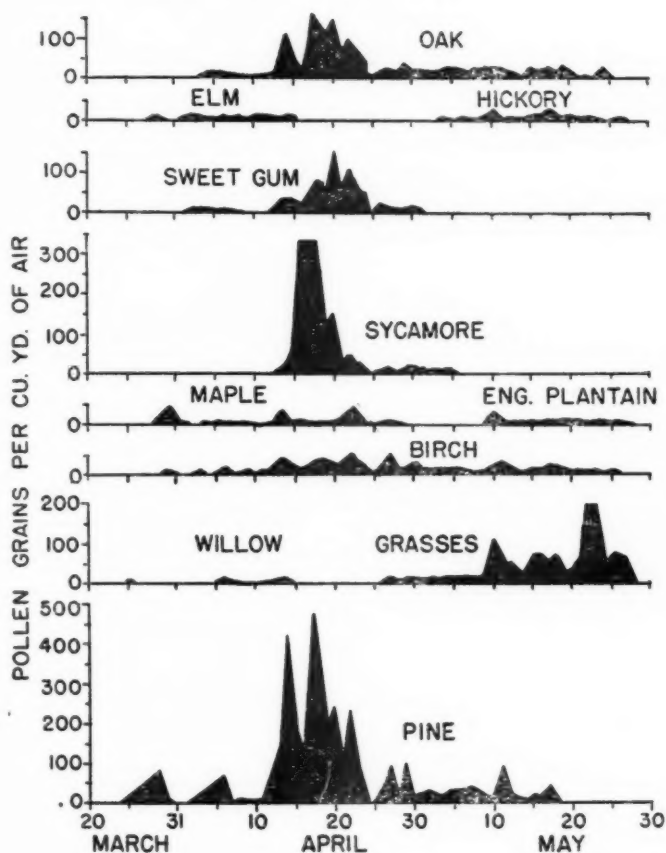
The leucopenic index devised by Dr. Vaughan adds another important diagnostic method to our list. The technique is as follows: The patient reports to the office in the morning in the fasting state. Two fasting white counts are made at ten minute intervals. The patient then eats an average portion of the food to be tested. Subsequent white counts are made at fifteen minute intervals for one hour. A final white count is made at the end of an hour and a half.

Frequently it becomes necessary to make up an autogenous dust extract. A cigar box full of dust, preferably from the vacuum cleaner, is sufficient. Add enough Coca's solution to make a thick mush and overlay with an ounce of toluol. Growth of the enormous number of bacteria in the dust is inhibited by the toluol. Cover tightly and let stand for 48 hours. Strain through a double layer of gauze and then filter through filter paper. The solution is now ready to be sterilized by filtration through the Seitz or Berkfeld filter and then culture for sterility. It is impossible to standardize a solution of this sort since the allergenic element is an unknown quantity.

In our laboratory we prepare all pollen extracts used for the diagnosis and treatment of a patient. The dried pollen is secured from a commercial house and 2% solution is made with Stier-Hollister. Allow the preparation to stand at room temperature with occasional shaking for 48 hours. Filter first through filter paper and then through the Seitz filter. Extracts of pollen made by this method are quite stable and the glycerin prevents contamination of the extract by air bacteria. This preparation is unsuitable for the ophthalmic test.

Probably the most fascinating and interesting duty of the allergy technologist is the daily pollen count and botanical surveys of the wind-borne pollens. The pollen is caught by exposing slides coated with methyl-green glycerin jelly. The glycerin jelly as described by Wodehouse is prepared by the method of Brandt which

ATMOSPHERIC POLLEN SURVEY OF NEWPORT NEWS, VA.
MARCH 25 TO MAY 28, 1937.



is as follows: Soak a fine grade of gelatin for 2 or 3 hours in cold water, pour off the superfluous water, and heat until melted. To one part of this add $1\frac{1}{2}$ parts glycerin and while still hot, filter through spun glass pressed into the lower part of a heated funnel. Add phenol to make a 2 or 3 per cent solution. Keeping the mixture hot, add drop by drop a saturated solution of methyl green in 50% alcohol until the glycerin jelly becomes as dark as green ink. The proportions of glycerin and water are so balanced that the pollen grains will be fully but rarely over-expanded. A small drop of this melted jelly is placed on a slide and spread out to occupy an area the size of the cover glass to be used in the finished mount. The slide is exposed in the horizontal position and protected from rain and sun by a shelter at least 4 inches above it. After 24 hours exposure the slide is brought to the laboratory and all extraneous materials such as sand and soot are removed with forceps. The slide is heated gently, controlling the temperature with the back of the hand, to drive off any excess moisture, and the cover glass fitted in position. If the slide has dried out too much add an additional drop of melted jelly.

The pollen count is made by examining under the microscope and counting the number of grains found in an area of 1.8 square centimeters of slide. This expresses the number of pollen grains in a cubic yard of air.

The most practical way of learning to recognize the pollens is to make mounted specimens of the different species. This may be done by going directly to the plants and collecting fresh specimens or using the dried pollen bought commercially. These are kept in the laboratory and referred to frequently for size and contour of the different pollen grains.

The tree pollens are the most difficult to identify. No attempt was made to differentiate the grasses. They can however be separated out to some extent by field observations of their flowering periods.

The most important hay fever producing plants are the rag-weeds. We have made no attempt to identify the different species of ragweed pollen caught on the slides.

Summary

1. It has been my desire to simplify this subject as much as possible. I have presented the technique for carrying out the cutaneous and intradermal tests, the preparation of the extracts, and the precautions necessary to take. I have also reviewed the

ophthalmic, patch, leucopenic index, and passive transfer tests.

2. I have shown a method for making daily pollen counts and also a method of mounting the pollen grains.

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EDITORIAL

TO THE REGISTERED MEDICAL TECHNOLOGISTS OF AMERICA

There has apparently been some confusion and a good deal of misunderstanding concerning the various Technologists' organizations now in existence, and it is my purpose to try to throw some light on the matter by giving you a brief outline of those Societies, which should be of interest to you, and therefore clarified for you.

I. *The Registry of Medical Technologists* is not a Society. It is a central office where Technologists with the proper prerequisite training, may obtain, upon passing an examination a certificate showing that they are qualified to practice the profession of Medical Technology. The Registry is conducted by a Board of Registry consisting of six Clinical Pathologists elected by the American Society of Clinical Pathologists. Applications for becoming registered may be sent to Mrs. Anna R. Scott, Registrar, 234 Metropolitan Building, Denver, Colorado. The examination fee is \$10.00 and the yearly fee for certificate renewal is \$1.00.

II. *The American Society of Medical Technologists*, is a Society made up of Registered Technologists only. To belong to this Society you must first become Registered. This Society was founded in Chicago in 1933 and holds annual conventions at the same time and place as the American Medical Association. In this way its members have the benefits of highly instructive and educational exhibits and the opportunity of hearing excellent speakers.

The objects of this Society, according to its present constitution are to

- a. Promote higher standards in clinical laboratory methods and research
- b. Elevate the status of those specializing in laboratory technique
- c. Create closer co-operation between the physician and the Technologist

EDITOR'S NOTE: *This editorial was prepared by F. H. Claussen, Chas. T. Miller Hospital, St. Paul, Minn., Past President of the American Society of Medical Technologists.*

Application for membership in this Society should be made to John H. Conlin, Administrative Secretary, 10439 East Outer Drive, Detroit, Michigan. The initial fee is \$1.00 and dues are \$4.00 a year, which dues include a subscription to the Journal issued every two months.

It seems fitting at this point to put in an extra word of appeal to Registered Technologists who have not yet joined this Society. Out of the five thousand R.M.T.'s in this country only about five hundred have shown the initiative and interest to join together in a common cause for the good of their profession. All other professions are way ahead of us. The X-Ray Technicians, the Social Service Workers, the Hospital Librarians, the Physiotherapists and Occupational Therapists, all these have organizations far exceeding ours in numbers and in strength.

In studying the diagnosis of the slowness of the growth of our Society, I can attribute it to three things only.

1. Many Technologists have confused the Registry with the American Society, do not understand the difference between the two and think that they belong to the latter when they receive their registration card. This became especially evident at the last convention in Atlantic City, when dozens of R.M.T.'s came to the meetings of the A.S.M.T. thinking they were members. They were welcome as guests, but were unable to vote at the business sessions or election of officers.

2. Many Technologists do not seem to know of the existence of the American Society at all. The Society has attempted to advertise itself by sending one issue of its Journal each year to the Registered Technologists who are non-members. This has been done at great expense to the Society and has brought in some new members, but what we need is personal contacts, and we appeal to each one of you who may read this article to pass on its explanation to your Technologist friends.

3. Many Technologists object to the \$4.00 dues, arguing that it is too much to pay for what they receive in return. If all the five thousand Registered Technologists would join the American Society, the dues might soon be lowered, and the ambitious and conscientious few would not be obliged to pay for the benefits that the many will undoubtedly eventually reap. If you will take the time to inquire as to the dues in other Societies and other professions, I am sure you will find that \$4.00 is not an unreasonable amount to pay toward the advancement of your own profession, even though you might be unable to attend the conventions yourself.

In these troublesome times of labor unions and strikes it is imperative that we Registered Medical Technologists work together 100%.

The Clinical Pathologists are doing all they can to help us raise the standards of our schools, and of our positions, and to further our post graduate education. They cannot do everything for us—we must do something for ourselves if we are ever to progress in the medical world.

Keep abreast of the times. Know what is going on in the field of technology. Read the papers that other Technologists are writing. Hear what other Technologists are doing. Keep in contact with your fellow workers and share your problems with them. That is what Societies are for, and whether you are isolated in a small town, or are one of a large laboratory force of workers, you are not self-sufficient, you need what this Society has to offer.

Phyllis Stanley, Chairman of the Advisory Board of the A.S.M.T., stands ready and willing to answer your questions or to help you with your problems. Her address is Presbyterian Hospital, Newark, N. J.

III. *The State Societies of Medical Technologists.* Many state societies have already been formed and many are just in the forming. Each society is, of course, regulated by its own constitution and the dues and regulations naturally vary in different parts of the country. It is the object of the American Society to eventually affiliate all the state societies, though this naturally takes some time for its development. When the affiliation rules and regulations have been perfected and incorporated into the Constitution of the American Society, it will undoubtedly be feasible to work out some sort of a delegate system, by which the problems and business of the Society and its affiliated members may be expedited, leaving more time at the conventions for scientific and social contacts. State societies should be made up of Registered Technologists only, in order to become affiliated with the National Society.

I would urge you, too, to join your state society, for your own State meetings will undoubtedly be of greatest value to you.

IV. *Local Societies.* These, of course, are so varied in make up and regulations that it is impossible to discuss them here. They are closest to you, and you may think they mean the most to you, but they cannot give you the broadening, strengthening influence of the National Society. It is well to know the Technologists of your own city, and State, but especially gratifying and instructive to meet Technologists from various parts of America.

Probably if you totalled the dues of your Local, State and National Society, plus your Registry certificate renewal you would find that the cost would be well under \$10.00 a year—less than \$1.00 a month—less than 25c a week. Do you want to be a good Technologist, well versed in current problems, well up on new methods? Do you want to help yourself and future Technologists be somebody and amount to something? Do you want your profession to be well known, and nationally recognized and honored and respected?

If a thing is worth doing at all, it is worth doing well. Why not do your bit in making the name "Registered Medical Technologist" mean something to the world?

ABSTRACTS

SPLENOMEGALY WITH MYELOID TRANSFORMATION: G. R. Tudhope. Jour. Path. and Bact., January, 1937.

Report of a case of gross splenomegaly which on autopsy showed myeloid transformation of the splenic pulp and marked anatomical changes in the liver and bone marrow, yet gave a blood picture of a moderate secondary anemia with immature cells absent.

THE INFLUENCE OF COPPER IN PEPTONES ON THE GROWTH OF CERTAIN PATHOGENS IN PEPTONE BROTH: R. A. Q. O'Meara and J. C. Macsween. Jour. Path. and Bact., Jan., 1937, p. 225.

The amount of free Cu in commercial peptones varies greatly. Representative organisms of different groups were grown in copper-free broth and in broth containing approximately that introduced ordinarily. Pyogenic cocci, the corynebacteria and various other aerobic and anaerobic organisms were found to be inhibited while the Gram negative intestinal bacteria showed much greater resistance.

Even where it does not inhibit, it may affect the growth characteristics such as pellicle formation, etc.

Addition of serum was found to neutralize the inhibitory effect of the copper.

A COLORIMETRIC METHOD FOR THE DETERMINATION OF SERUM MAGNESIUM BASED ON THE HYDROXYQUINOLINE PRECIPITATION: W. S. Hoffman. Jour. of Biol. Chem., March, 1937, p. 37.

The author gives a new method for the determination of serum magnesium which may be read colorimetrically or by means of a photo-electric colorimeter.

ANALYSIS OF A CASE OF PERNICIOUS ANEMIA WITH RETAINED SECRETION OF HYDROCHLORIC ACID AND WITH PRESENCE OF CASTLE'S INTRINSIC FACTOR IN THE STOMACH: K. Wolf and F. Reimann. Z. Klin. Med., 130, 789-807 (1936).

The author suggests that pernicious anemia may be based on an impermeability of the gastrointestinal wall to the antianemic factor.

BOOK REVIEW

LEGAL MEDICINE AND TOXICOLOGY, by Thomas A. Gonzales, MD., Acting Chief Medical Examiner of the City of New York; Associate Professor of Forensic Medicine, New York University College of Medicine; Lecturer on Criminological Medicine, New York Police Academy; Morgan Vance, M.D., Assistant Medical Examiner of the City of New York; Assistant Professor of Forensic Medicine, New York University College of Medicine; Lecturer in Forensic Medicine, College of Physicians and Surgeons, Columbia University; Lecturer on Criminological Medicine, New York Police Academy; and Milton Helpern, M.D., Assistant Medical Examiner of the City of New York; Assistant Professor of Forensic Medicine, New York University College of Medicine; Lecturer in Legal Medicine, Cornell University Medical College; Lecturer on Criminological Medicine, New York Police Academy. Published by the D. Appleton-Century Co., 35 West 32nd Street, New York City. Pp. 754. Price \$10.00.

This book is divided in the usual manner into two main sections, that of legal medicine and of toxicology. In the former the authors have drawn from abundant material in the City of New York where about 75,000 deaths occur annually and about 15,000 of these are investigated by the medical examiner's office. This part is profusely illustrated with photographs taken at the scenes of crimes and at the autopsy table. All phases of medico-legal investigation are considered beginning with the duties of the coroner and medical examiner, identification, technique of the autopsy, signs of death, etc., to a detailed discussion of all the various kinds of trauma that cause death. Other chapters are devoted to the medico-legal aspects of pregnancy, puerperium, illegitimacy, abortion, sexual assaults and infanticide. The microscopic examination of hair and other minute objects, medico-legal examinations of blood and the iso-agglutination tests for human blood are duly considered. Short chapters are given over to the medico legal aspects of the various forms of insanity, the rights and obligations of physicians and insurance, survivorship and malingering.

The section on toxicology gives a thorough discussion of the usual and many of the unusual organic and inorganic poisons, some of which are not included in most other works on toxicology. The last three chapters of about 100 pages takes up toxicologic analysis.

The book is thorough and authoritative. The authors write largely from an extensive practical experience in dealing with the

medical problems presented by the crimes of the second largest city in the world. The book is well written and the subject matter presented in logical form. It should be of particular value to every coroner, county physician and medical examiner, the toxicologist and pathologist and any laboratory assistant or technician who may be called upon to aid in the investigation of the so-called coroner's case.

NEWS AND ANNOUNCEMENTS

REGISTRY OF MEDICAL TECHNOLOGISTS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The April, 1937, examination was conducted throughout the United States and Canada. There were three hundred and sixty-nine successful applicants and fifty-two who did not pass the tests.

The Examining Board used the following questions in the written test:

1. Give the technic for the preparation of two-thirds normal sulphuric acid and N/10 sodium hydroxide.
2. Classify the blood groups according to the Moss, Jansky and International systems.
3. *A.* Name four methods for the determination of hemoglobin. Give the technic for one of the acid-hematin methods.
B. What is the routine dilution of blood cells in the leukocyte pipette? In the erythrocyte pipette? What is the measurement of the smallest division of the ruled area of the counting chamber?
4. Define the following:
 - (a) Heterophile antibody test;
 - (b) Fragility test for erythrocytes;
 - (c) Icterus index
 - (d) Myeloblast
 - (e) Neutropenia
 - (f) Volume index
 - (g) Sedimentation time
 - (h) Thrombopenia
 - (i) Leukemia
 - (j) Polycythemia
5. *A.* Give the technic for either the P. S. P. test or the concentration dilution test for urinary function.
B. Name four conditions which may cause the cloudiness of the urine and explain how to demonstrate them.

6. Give the normal figures for the following:
 - (a) Blood sugar
 - (b) Total non-protein nitrogen content of blood
 - (c) Blood chlorides
 - (d) Blood creatinine
 - (e) Blood cholesterol
7. What is the technic for a glucose tolerance test?
8. Name the organisms used in the serum agglutination tests for:
 - (a) Undulant fever
 - (b) Typhus fever
 - (c) Typhoid fever
9. *A.* List the laboratory procedures used for the diagnosis of tuberculosis.
B. How would you differentiate pneumococcus?
10. *A.* Describe the Widal reaction.
B. Broadly define:
 - (a) antigen
 - (b) complement
 - (c) amboceptor, and give a method of obtaining each (without reference to the Wassermanns)

NATIONAL

The new issue, of membership certificates, is now being sent to active members as dues are recorded at the administrative office.

Indiana

The Indiana Society of Medical Technologists held their second annual meeting at the City Hospital in Indianapolis June 18-19, and the following program was presented:

Friday, 2 P.M.—Hematology—Dr. C. G. Culbertson, Assistant Professor of Pathology, Indiana University. 8 P.M.—Some Simple Tests for Poison—Dr. R. N. Harger, Professor of Toxicology, Indiana University.

Saturday, 9 A.M.—Some Widal Observations—Marjorie Joiner, Eli Lilly Laboratory. Round Table Discussion of Serology Problems. Election of Officers. Luncheon Meeting, Methodist Hospital. 2:30 P.M.—Prophylaxis and Therapy in Bacterial Infections—Dr. G. F. Kempf, Lilly Laboratory for Clinical Research. 3:30 P.M.—Preparation of Allergy Extracts—Alice Miller, City Hospital Lab. Round Table Discussion of Bacteriology Problems. 7:00 P.M.—Banquet.—Speaker Dr. L. H. Segar. "Interesting Children From a Medical Standpoint."

